Pheromone variability and evolution in the butterfly genus *Bicyclus*, and implication in its diversification.

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Abstract

The evolution of olfactive communication in generating reproductive isolation among species remains poorly understood (Smadja & Butlin 2009). In Lepidoptera, studies have mainly focused on long-distance pheromones produced by moths. Moth sex pheromones have been shown to display inter-population variation (e.g. Tòth et al. 1992, McElfresh & Millar 2008 and ref. within, Groot et al. 2009) and to be involved in interspecific isolation (e.g. Löfstedt et al. 1991, Groot et al. 2006). In butterflies, the few existing studies on sex pheromones have mainly focused on the identification of the male specific compounds and the demonstration of their behavioural activity in courtship (e.g. Grula et al. 1980, Nieberding et al. 2008, Yildizhan et al. 2009), but have failed so far to highlight a role in reproductive isolation (Friberg et al. 2008).

In the species-rich *Bicyclus* genus Kirby, 1871 (Nymphalidae, Satyrinae) the structures producing the pheromones, i.e. the androconia, are key characters to discriminate among species (Condamin 1973). In *B. anynana* (Butler, 1879), the male sex pheromone (MSP) has been shown to play a role in mate choice (Costanzo & Monteiro 2007, Nieberding et al. 2008), to be heritable, and particular ratios of the pheromone components are under strong sexual selection (Nieberding et al, unpubl. data). Therefore, we expect that pheromone evolution is responsible for reproductive isolation and diversification in this butterfly group. In this framework, our research project aims at understanding the evolution of MSP at the interspecific level across the *Bicyclus* genus and specifically at testing their potential role in the speciation process.

Potential MSP of several species across the *Bicyclus* genus have been identified by gas chromatography and mass spectrometry. Observed differences in pheromone composition between species are compared in a phylogenetic framework to the molecular tree of the

species (following Oliver et al. 2009). We expect the evolutionary rate of MSP to be unlinked to the molecular tree if MSP are under sexual selection across the genus (i.e. saltational evolution following Symonds & Elgar 2004, Shirangi et al. 2009). Moreover, if MSP generated reproductive isolation between species in a “reinforcement” process, we expect higher differences of MSP composition between sympatric species than between allopatric species and an increase of this pattern for younger species compared to older species (Lukhtanov et al. 2006).

Keywords : Male sex pheromones, gas chromatography, mass spectrometry, comparative analysis, phylogenetics, speciation, *Bicyclus*.

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